J. Plant Biochemistry & Biotechnology Vol. 7, 51-52, January 1998

Short Communication

Differential Expression of Uptake Hydrogenase Activity in Free Living Cells and Nodule Bacteroids of *Azorhizobium caulinodans*

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An oxygen sensitive mutant of *Azorhizobium caulinodans* strain IRBG 46 in which N_2 fixation ability was affected, was previously isolated by NTG mutagenesis. Now, the mutation has been shown to affect H_2- uptake hydrogenase (Hup) activity under symbiotic conditions. However, free living Hup activity remained unaffected. Thus the mutant is Hup$^-$ under symbiotic conditions and Hup$^+$ under free living condtions. A possible regulatory link between N_2 fixation and H_2 uptake system has been discussed.

Key words : oxygen sensitive, *Azorhizobium caulinodans,* uptake hydrogenase, differential regulation.

Nitrogenases from all known sources have an inherent property of evolving molecular hydrogen concomitant with the reduction of dinitrogen to ammonia. Many aerobic N_2-fixing bacteria, including cyanobacteria, azotobacters and rhizobia, are capable of expressing a hydrogenase that oxidizes H_2 generated by the nitrogenase complex. Among the root nodule bacteria, H_2- uptake hydrogenase positive (Hup$^+$) strains have been found in species of the genera *Rhizobium, Bradyrhizobium* and *Azorhizobium. Azorhizobium caulinodans* assumes a unique position amongst the nitrogen fixing organisms because of its ability to fix N_2 in aerial stem nodules as well as in free living cells at a relatively high oxygen concentration (1, 2). Moreover, it has an efficient H_2 recycling system which unlike all other rhizobia can be derepressed for Hup activity in the presence of high organic carbon under free living conditions (3). As in expression of nitrogen fixation genes, oxygen has also been shown to regulate the expression of Hup activity in chemoautotrophically induced cell suspension of rhizobia (3, 4). Earlier we have studied the oxygen protection mechanism in diazotrophic *A. caulinodans* strain IRBG 46 by developing an oxygen sensitive mutant (5). In this communication we suggest a role of some common regulatory factor(s) in N_2 fixation and H_2 uptake system.

In this study *Azorhizobium caulinodans* strain IRBG 46 and its oxygen sensitive mutant C48 isolated earlier by NTG mutagenesis (5), were employed. In order to see the effect of the mutation on expression of *hup* genes, H_2 uptake assay was carried out under both free living and symbiotic conditions. Free living Hup assay was done as described by Sanghi and Lodha (6). For symbiotic assay, plants were grown under natural conditions in plastic pots (5). Nodules from stem and roots were collected separately in 25.5 ml glass vials provided with serum stoppers. These were incubated for 1h at 30°C. One ml gas sample was withdrawn from each vial and its H_2 content was analysed by gas chromatograph.

It can be seen from the Table 1 that free living cells of both the parent strain IRBG 46 and its mutant C48 were Hup$^+$ under atmospheric O_2 level as well as microaerobic condition. However, under symbiotic condition, while the parent strain did not evolve H_2, the mutant evolved H_2 (Table 2). These results indicate that the mutant C48 is Hup$^+$ under free living conditions, but Hup$^-$ under symbiotic conditions. This means, the mutation might have affected some gene(s) which are involved only in symbiotic expression but not in free living expression of Hup activity.

Table 1. Uptake hydrogenase activity of free living cells of *A. caulinodans* strain IRBG 46 and its oxygen sensitive mutant C48 under 1% and atmospheric O_2 levels

Strain	Sp uptake hydrogenase activity (nmol H_2 h^{-1} mg^{-1} protein)
Atmospheric O_2 level	
IRBG 46	7663 ± 524.4
C 48	6713 ± 384.6
1% O_2 level*	
IRBG 46	3229 ± 380.0
C 48	3169 ± 329.7

*in N_2 atmosphere.
Note : Values reported are mean ± SE of six replicates.

Thus, in *A. caulinodans* there is differential expression of *hup* genes in free living cells and symbiotic bacteroids. Similar findings were reported by Palacios *et al* (7) in *R. leguminosarum.* In this bacterium out of six *hup*

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Table 2. H_2 evolution rates in the root and stem nodules of *Sesbania rostrata* infected with *A. caulinodans* strain IRBG 46 and its oxygen sensitive mutant C 48

Strain	Nodule dry wt (mg plant^{-1})	H_2 evolution rate (μmol H_2 h^{-1} g^{-1} nodule dry wt)
Root nodules		
IRBG 46	43	nd
C 48	19	53.3 ± 7.71
Stem nodules		
IRBG 46	46	nd
C 48	23	35.1 ± 15.78

Note : Values reported are mean ± SE of three replicates. Each replicate consisted of 3 plants for IRBG 46 and 4 plants for the mutant.

transcriptional units (*hup* I to *hup* VI) only two (*hup* V & *hup* VI) were expressed in free living cells under microaerobiosis. However, in bacteroids all six transcripts were expressed. They suggested that expression of *hup* I to *hup* IV may require in addition or independently a specific plant factor or particular combination of environmental conditions found only in the nodules, but not in free living cells. Here, in IRBG 46 we suggest that there may be a specific transcriptional activator which is expressed only in nodule bacteroids in response to above signals. Mutation might have affected a gene whose product positively regulates the expression of the putative transcriptional activator or act in concert with the transcriptional activator to activate *hup* genes under symbiotic conditions. In *Bradyrhizobium japonicum* a transcriptional activator was identified (8, 9). However, the situation in *B. japonicum* is reverse since this potential transcriptional activator is involved in free living expression of *hup* genes. It has also been suggested that there may be a common regulatory element(s) controlling the expression of *nif* and *hup* genes by O_2 (7). In *R. meliloti* the common regulatory element(s) has been found to be FixLJ. Since in *A. caulinodoans* the mutation possibly located on *fix*L or *fix*J genes, has been found to affect the expression of nitrogen fixation genes(5), a similar role of FixLJ as a common regulatory element of *nif* and *hup* gene expression can not be ruled out.

Acknowledgement

The senior author thanks the Director, Indian Agricultural Research Institute for providing SRF during the tenure of his study.

Received 21 November, 1997.

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